BACTERIAL EXAMINATION OF WATER

The bacteriological examination of water is performed routinely by water utilities and many governmental agencies to ensure a safe supply of water for drinking, bathing, swimming and other domestic and industrial uses. The examination is intended to identify water sources which have been contaminated with potential disease-causing microorganisms. Such contamination generally occurs either directly by human or animal feces, or indirectly through improperly treated sewage or improperly functioning sewage treatment systems. The organisms of prime concern are the intestinal pathogens, particularly those that cause typhoid fever and bacillary dysentery.

Since human fecal pathogens vary in kind (viruses, bacteria, protozoa) and in number, it would be impossible to test each water sample for each pathogen. Instead, it is much easier to test for the presence of nonpathogenic intestinal organisms such as *E. coli*. *E. coli* is a normal inhabitant of the intestinal tract and is not normally found in fresh water. Therefore, if it is detected in water, it can be assumed that there has been fecal contamination of the water.

In order to determine whether water has been contaminated by fecal material, a series of tests are used to demonstrate the presence or absence of coliforms. The **coliform** group is comprised of **Gram-negative, nonspore-forming, aerobic to facultative rods, which ferment lactose to acid and gas.** Two organisms in this group include *E. coli* and *Enterobacter aerogenes*; however, the only true fecal coliform is *E. coli,* which is found only in fecal material from warm-blooded animals. The presence of this organism in a water supply is evidence of recent fecal contamination and is sufficient to order the water supply closed until tests no longer detect *E. coli.* Total coliforms respond in a manner similar to most bacterial pathogens and many viral enteric pathogens, thus they are considered a useful indicator of bacterial and viral pathogens in water. Total coliforms originate as organisms in soil or vegetation and in the intestinal tract of warm-blooded animals (e.g., *E. coli*).

In this exercise, you will be testing water samples for the presence of coliforms. We will be using the membrane filtration (MF) method for the detection and enumeration of total coliform bacteria to achieve this goal.

THE MEMBRANE FILTRATION METHOD

This procedure using membrane filters has now been recognized as a reliable method for the detection of coliforms in water. Parallel testing has shown that results obtained from the membrane filtration method are equivalent to those obtained by the multiple-tube tests. Standard methods for the bacteriological examination of water are detailed in *Standard Methods for the Examination of Water and Wastewater*, published by the American Public Health Association.

The membrane filter method is a direct plating method for the detection and enumeration of the coliform group of indicator organisms. Samples of water are passed through filter discs which have pore sizes of 0.45 μm diameter. Bacteria larger than 0.45 μm will be retained directly on the surface of the filter. The filter is then placed on a selective/differential medium such as M-Endo agar. The only carbohydrate in M-Endo agar is lactose. Lactose fermenters such as *E. coli* and *Enterobacter aerogenes* which are retained on the filter will form colonies with a characteristic greenish-golden metallic sheen within 24 hours of incubation. The coliform group, as defined by the production of aldehydes from fermentation of lactose, may result in variations in degree of metallic sheen development among coliform strains.

The size of the sample to be filtered is governed by the expected bacterial density. An ideal quantity will result in the growth of about 20 to 80 coliform colonies and not more than 200 colonies of all bacterial types. Typical coliform colonies are counted and their density is reported in terms of (total) coliforms per 100 ml of sample.

The advantages of this method over multiple-tube tests are: (1) higher degree of reproducibiltiy of results; (2) greater sensitivity, since larger volumes of water may be tested; and (3) shorter time for obtaining definite results. There are, however, certain limitations on the use of the membrane filter technique. Its effectiveness is decreased in water samples characterized by high turbidity, high concentrations of heavy metals or the presence of toxic materials such as phenols. Turbidity caused by the presence of algae or other interfering material may not permit examination of a sample volume large enough to yield significant results. Toxic substances, on the other hand, may cause low coliform estimates.

The City of Bellingham water treatment plant laboratory uses the total coliform MF method to analyze samples such as raw source water, new construction, customer service requests, and special projects that require the enumeration of total coliform bacteria. In addition, if a drinking water distribution system sample result is positive for total coliform all follow up tests will include analysis using the MF method. It is necessary to filter 100 milliliter (ml) of sample when analyzing drinking water for compliance reporting. Sample size of non-drinking water samples is determined based on the expected bacterial density of the water source.

FIRST PERIOD

Material:

1. Vacuum filtration manifold set-up for 47-mm filters
2. Vacuum pump (12 to 15 psi)
3. Sterile foil-wrapped calibrated filtration funnel
4. Sterile 47 millimeter (mm) diameter 0.45 micrometer (μm) pore size membrane filters with grids.
5. Sterile forceps (dip into ~70% ethyl alcohol and then flame)
6. Sterile cotton-plugged disposable pipettes
7. mEndo agar plates (smaller size, 60mm plates)
8. Sterile 0.1 % peptone solution
9. Water samples

Procedure: (work in groups of four)

1. Select sample volumes attempting to yield ~20 to 80 colonies on the membrane surface. This will be done by used 100 ml undiluted, 50 ml, 10ml, 5 ml and 1ml samples (i.e., four dilutions with peptone solution).
2. Label all plates with the sample location and dilution.
3. Set up the filtration funnel.
4. Load a sterile membrane, grid side up, onto the center of the porous part of the filter support base. Handle membrane filter with flamed forceps.

5. Thoroughly mix sample by shaking up and down from shoulder-height to elbow-height 25 times in long sweeping movements.
6. Aseptically filter the sample (use most dilute sample FIRST):
   A. For sample volumes 50 to 100 ml pour directly into the calibrated funnel.
   B. For sample volumes 10 to 49 ml use a sterile pipet to transfer the sample into the funnel.
   C. For sample volumes less than 10 ml first pour 20 to 30 ml peptone solution into the funnel then pipet the sample into the peptone.
7. Turn on the vacuum and filter the sample.
8. Rinse the walls of the funnel three times with 20 to 30 ml of peptone.
9. Turn off the vacuum and allow pressure to equilibrate.
10. Remove filter with sterile forceps and roll onto mEndo agar being careful to avoid trapping air bubbles.
11. After filtering samples, invert plates and incubate all plates at **35ºC** for **22 - 24 hours**. Either count plates right away or save till second period.

SECOND PERIOD

Material:

1. Stereo Microscope - 10x to 15x magnification (with an external light source).

Procedure:

1. Count colonies using a low power magnification (10x to 15x) microscope, with a fluorescent light source adjusted at an angle 60 to 80 degrees above the colonies.
2. Typical colonies have a pink to dark red color with a metallic greenish-yellow sheen. Atypical (non-sheen) colonies vary in appearance from colorless to deep red with no metallic sheen.
3. Count plates with fewer than 200 colonies. Count the number of typical and atypical colonies on each plate and record these results to two significant digits.
4. Remember to report typical and atypical as well as total coliforms as CFUs per 100 ml.

THE CHROMOGENIC SUBSTRATE TEST

Simple one-step defined substrate tests for detecting coliforms are now available. These tests are designed to detect the presence or absence of coliform bacteria and to indicate specifically the presence or absence of *E. coli*. The Colilert® system is one example of a P-A (presence-absence) test. A water sample is added to a special medium containing **ONPG** (*o*-nitrophenyl-β-D-galacto-pyranoside) and **MUG** (4-methylumbelliferyl-β-D-glucuronide). These substrates are the major sources of carbon in Colilert®. **ONPG** is hydrolyzed by β-galactosidase, the enzme that cleaves lactose to glucose and galactose. The medium will turn yellow if coliforms, which have the ability to ferment lactose, are present. *E. coli* uses another enzyme, β-glucoronidase, to metabolize **MUG**. The modified **MUG** yields a fluorescent product that can be seen under long-wavelength ultraviolet light. Non-target organisms, i.e. non-coliforms, are both starved and suppressed in the Colilert® medium. Refer to the attached AWWA report for more information.

FIRST PERIOD

Procedure: (work in groups of four)

1. Add 100 ml of the water sample to a sterile, transparent, non-fluorescent bottle provided by Colilert®.

2. Tap one of the "snap packs" to ensure that all of the Colilert® reagents are in the bottom part of the pack. Pour contents of "snap pack" into the water sample. Cap and seal the bottle, and shake until the Colilert® reagents have dissolved.

3. Incubate the bottle for **22- 24 hours at 35°C**.

SECOND PERIOD

1. Read the results at no more than **24 hours**. Compare each result against the "comparator" provided by Colilert®.

- ☞ If no yellow color is observed, the test is negative.
- ☞ If the sample has a yellow color equal to or greater than the "comparator", the presence of coliforms of confirmed.
- ☞ If the sample is yellow, but lighter than the "comparator", incubate for 4 more hours (but no more than 28 hours total). If coliforms are present, the color will intensify. If the color does not intensify, coliforms are absent.
- ☞ If the sample developed a yellow color, check for fluorescence. If fluorescence is greater to or equal to the fluorescence of the "comparator", the presence of *E. coli* is confirmed.

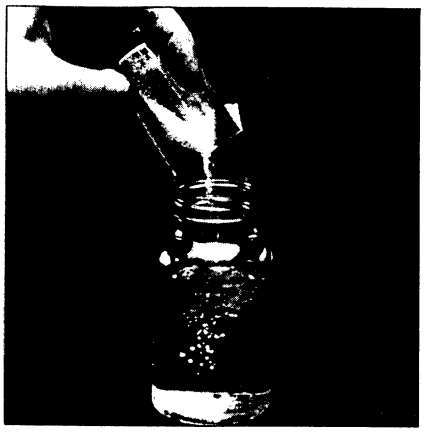
NUMBER 4 AWWA Research Foundation MAY 1993

RESEARCH APPLICATIONS

PROVEN UTILITY BENEFITS

American Water Works Association RESEARCH FOUNDATION

Improving Bacterial Analysis While Lowering the Cost



Adding ready-to-use Colilert reagent to a water sample

Problem: Ensuring that drinking water is free of infectious organisms is a prime objective of the water supply community. Water purveyors have historically used microbiological analysis methods such as multiple tube fermentation and membrane filtration to measure bacteriological quality of water.

Since it is impossible to monitor for all human pathogens, these methods rely on the detection of indicator organisms, such as total or fecal coliform bacteria. Indicator tests signal when drinking water quality may be compromised.

However, *Escherichia coli* is a better indicator of contamination of public health significance. Without a simple method to speciate *E. coli*, water purveyors have had to rely on less specific, more time consuming, and more complex methods to test microbiological quality.

Solution: A technology used to identify *E. coli* and total coliform bacteria in clinical microbiology was adapted for the drinking water industry by Stephen Edberg at Yale University's School of Medicine. This technology, termed Colilert®,* was evaluated through a Research Foundation project. The project field validated Colilert's ability to simultaneously enumerate total coliforms and *E. coli* from drinking and source waters without the need for confirmatory tests.

The test uses simple equipment—an incubator and ultraviolet light (366 nm). Addition of the ready-to-use reagent to water samples in test tubes or culture bottles results in a colorless solution. The solution is incubated at 35°C for 24 hours. A yellow color indicates the presence of total coliforms, and fluorescence indicates the presence of *E. coli*.

The original research project, which consisted of a national evaluation of the method, cost $170,000 in AWWARF and in-kind funds. The research was subsequently validated by the U.S. En-

*Colilert is a registered trademark of Environetics, Inc., Branford, Conn.

TABLE 1

Cost Savings for Materials, Labor, and Quality Control*

	Utility Labs	Private Labs	Public Health Labs
Cost per Membrane Filtration Test	$4.40-$5.80	$4.10-$4.70	$5.80-$6.60
Cost per Colilert Test	$3.20-$3.50	$3.20-$3.50	$3.20-$3.50
Savings+	$1.20-$2.30	$0.90-$1.20	$2.60-$3.10

*Averages based on information provided by laboratories certified for microbiological analyses

+Depends on the number of samples per month

vironmental Protection Agency (USEPA), the American Water Works Association, many states, and numerous utilities. It is a USEPA-approved method for total coliform and *E. coli*.

Colilert is valuable for routine monitoring by all sizes of water purveyors and water quality laboratories worldwide. In fact, 46 U.S. states have approved use of the test for drinking water compliance monitoring, and approval is pending in others. Colilert is being used in several Canadian provinces where it has or is expected to receive official approval.

Because it is fast and reliable, the Colilert method is also useful in water quality emergencies following hurricanes, earthquakes, and main breaks. For example, this method was used following the San Francisco earthquake, Hurricane Andrew in Florida, and a cholera outbreak in South America. And it is used for monitoring of rural water supplies in developing countries; for testing marine water, wastewater, private well water, and recreation water; and for U.S. military operations.

Benefits: This issue of *Research Applications* provides information on potential cost savings with Colilert. These cost savings are directly related to this method's ease of use, simplicity, and accuracy.

Colilert is generally less expensive to use than membrane filtration (see Table 1). The cost savings are based on information provided by utility and water laboratories certified for microbiological analyses. These public and private laboratories represent a variety of geographical locations and number of samples processed.

Because savings depend on the number of samples processed per month, a range of values is given. The higher figures represent savings for laboratories that process 200-300 samples per month. The lower figures indicate savings for more than 400 samples per month. The larger laboratories process so many samples each month that they realize some cost savings on materials regardless of the method used.

From the data in Table 1, a conservative estimate of annual savings for the water supply community alone is $13 million. In other words, this is an annual payback of more than $77 for each $1 invested in the original research project. The payback will continue to grow annually as more water utilities and laboratories use Colilert.

Colilert provides the water supply community with an analytical tool that will allow it to better monitor the microbiological quality of drinking water in a more efficient manner, ensuring the consumer with the highest quality water possible. The test's attributes will undoubtedly find favor with the water supply community and health organizations throughout the world in years to come.

More information available from the AWWA Research Foundation:

The Colilert® System for Total Coliforms and Escherichia coli. (Order number 90576.)

Colilert: A Better Method for Ensuring Drinking Water Quality. (To borrow a copy of this 14-minute VHS video, call (303) 347-6121.)

MA-205-93-5

Examples of Where Colilert is Used

- Emergencies
  - Cholera outbreaks
  - Earthquakes
  - Hurricanes
  - Storms and coastal flooding (marine water)
- Industry
  - Aquaculture (seafood estuaries)
  - Bottled water
  - Cosmetic
  - Dairy
  - Food and beverage
  - Pharmaceutical
- Military Operations
  - Kuwait restoration
  - Operation Desert Shield
  - Operation Desert Storm
  - U.S. naval vessels
- Remote and Field Testing
  - Afghanistan
  - Caribbean
  - Central and South America
  - Malawi
  - Sierra Leone
  - South Seas
- Source and Drinking Water Testing
  - Australia
  - Canada
  - France
  - Germany
  - Italy
  - Japan
  - New Zealand
  - Spain
  - United Kingdom
  - United States
- Transportation
  - Cruise ships
  - International airlines

American Water Works Association
RESEARCH FOUNDATION

6666 West Quincy Avenue
Denver, CO 80235
(303) 347-6100 ▪ Fax: (303) 730-0851

Subscribers can order reports directly from the Research Foundation by calling (303) 347-6100. Others can order reports from AWWA Customer Services by calling (303) 795-2449.

For further information, contact: Martin J. Allen, Director, Technology Transfer Division, (303) 347-6107; or Sharon C. Parks, Technology Transfer Specialist, (303) 347-6111.

FOOD MICROBIOLOGY

The presence of microorganisms in food is beneficial in some cases and harmful in other cases. Certain microorganisms are necessary in preparing foods such as cheese, pickles, sauerkraut, yogurt and sausage. Other microorganisms, however, may be responsible for serious and sometimes fatal food poisoning and toxicity as well as food spoilage (the product smells, looks, or tastes bad).

Microbial spoilage of any food depends on the chemical composition of the food and the types of organisms with which the food comes into contact. Consider a fresh Granny Smith apple containing a high percentage of carbohydrates. If a carbohydrate-fermenting organism came in contact with the inner tissue of the apple, the organism would survive, multiply, produce acid and gas (maybe alcohol), and, at the same time, destroy the tastiness of the apple. If a proteolytic or lipolytic organism came in contact with the same apple, the microorganism may not survive for long because of the non-availability of protein or lipids, and also because of the low pH of the apple tissue.

Two physical factors involved in the rate of food spoilage are the manner in which the food is processed and the method used to preserve the food. These include cooking, salting, drying, adding microbial inhibitors, adding sugar, canning, refrigerating, freezing and irradiating.

FIRST PERIOD

Material:

1. Samples of fresh raw hamburger, raw chicken, chicken salad, oysters, fresh unwashed vegetables, fresh unwashed fruits, dried fruits, cottage cheese, or creamy salad dressings
2. One 90-ml dilution bottle of sterile saline
3. Four 9-ml dilution tubes of sterile saline
4. Five nutrient agar plates
5. 1.0 and 0.1 ml pipets
6. Glass spreader
7. 95% ethyl alcohol in glass beaker

Procedure: (work in groups of four)

1. Add 10 g of the food product to be assayed into a Waring blender jar. Add 90 ml of sterile saline and blend the mixture at high speed until a uniform slurry is formed (approximately 1 to 3 minutes). You will have made a 10^{-1} dilution of the food sample.

2. Prepare serial dilutions (10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}) by transferring **1.0** ml at each step. Be sure to mix the diluted samples before each serial transfer.

3. Transfer **0.1** ml of each of the dilutions onto nutrient agar plates.

4. Incubate all plates in an inverted position for 2 days at 37°C.

SECOND PERIOD

Material:

1. Colony counter

Procedure:

1. Count the number of colonies on each plate and record.

Results:

Type of Food	PLATE	Number of Colonies	Number of Organisms per ml
	10^{-2}		
	10^{-3}		
	10^{-4}		
	10^{-5}		
	10^{-6}		